

Application No.: 10/090,965

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REMARKS

This Amendment is filed in response to the Office Action dated March 22, 2007.

Claims 1-13 are pending and stand rejected. The Examiner is requested to carefully review the present claims for any concerns so that this case may proceed to appeal, if needed, without such concerns being raised after filing of an appeal brief.

The Office Action is discussed below, followed by a review the Office Action's response to previously made arguments.

*The argument for obviousness in the Office Action*

Claims 1-13 stand rejected for obviousness in light of Madison et al., Johnston et al., Clemente et al., and Linde et al. The Office Action (at page 3) generally takes the position that Madison et al. suggests, at page 44 of Madison et al, improving polyhydroxyalkanoate (PHA) yields in yeast by increasing the activity of beta-ketothiolase and acetoacetyl-CoA reductase. The Office Action states that expressing enzymes transgenically is well known. Then the Office Action looks to Linde et al. and other art to support the idea that yeast are known to grow effectively in aerobic and anaerobic conditions such that one of ordinary skill in the art "would have recognized to use transgenic [yeast] under anaerobic or aerobic conditions, permitting flexibility in culture conditions and thereby improving cost effectiveness of producing PHA" (Office Action page 5). The Office Action also suggests that the motivation of producing PHAs under anaerobic conditions would be to increase efficiency of production of PHAs.

The Patent Office's record does not show support in the prior art for the proposition that growing yeast under aerobic and anaerobic conditions, permitting

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flexibility, would improve the cost effectiveness of producing PHAs. Please clarify the record on this point: how does permitting flexibility lead to cost effectiveness of producing PHAs; is this known in the prior art?

The Patent Office's record does not show support in the prior art that producing PHAs under anaerobic conditions would be to increase efficiency of production of PHAs. Please clarify the record on this point: how is it that producing PHAs would increase the efficiency of PHA production; is this known in the prior art? Does the improved efficiency come from better yields, or is this a referral to cost-savings?

*Traversal of the rejection*

The Office Action attempts to build a case for the obviousness and predictability of expressing beta-ketothiolase and acetoacetyl-CoA reductase in yeast in anaerobic conditions. But it fails to explain how the production of a certain metabolite, as claimed, would be predictable. Aerobic metabolism is not predictive for anaerobic metabolism.

Respectfully, the Office Action's position misses the Applicant's points. It is well known that switching a yeast from aerobic to anaerobic conditions *drastically* changes its *metabolism*. PHA is an important *metabolite*, i.e., it is the cell's *metabolism* that controls PHA production. Drastic changes in metabolism are expected to change the production of metabolites, such that it is altogether unpredictable as to whether or not the metabolites would be produced following any significant change, much less the enormous alteration of changing to anaerobic culture. Nothing in the Patent Office's record supports the proposition that a metabolite such as PHA would be unaffected by such a change.

These and other points have already been discussed in the record, which is partially reproduced, below.

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*An invention is not obvious when the relevant literature states that the achieving the invention is unpredictable.*

The prior art can be modified or combined to reject claims as *prima facie* obvious as long as there is a reasonable expectation of success. MPEP 2143.02. Obviousness does not require absolute predictability, however, at least some degree of predictability is required. Evidence showing there was no reasonable expectation of success may support a conclusion of nonobviousness. MPEP 2143.02. In the case Amgen v. Chugai, 927 F.2d 1200 (C.A.F.C. 1991) the court upheld a finding of nonobviousness since there would have been "no more than a fifty percent chance of success" in cloning the disputed EPO gene using prior art methods. 927 F.2d 1200, 1208. The court further pointed to the well established principle that "Both the suggestion and the expectation of success must be founded in the prior art, not in applicant's disclosure." 927 F.2d 1200, 1208. Even though it found that these procedures were "obvious to try," the references did not show that there was a reasonable expectation of success. 927 F.2d 1200, 1208, 1209, which also cited In re O'Farrell, 853 F.2d 894, 903-04, 7 USPQ2d 1673, 1680-81 (C.A.F.C. 1988).

In the present case, as discussed below, the literature states that these are unpredictable arts such that there would be no reasonable expectation of success. Therefore the rejection of the claims can not stand.

*An invention is not obvious when the cited references do no more than suggest it would be obvious to try to make the invention.*

"The admonition that "obvious to try" is not the standard under 35 U.S.C. §103(a) has been directed mainly at two kinds of error. In some cases, what would have been "obvious to try" would have been to vary all parameters or try each of numerous possible choices until one possibly arrived at a successful result, where the prior art gave either no indication of which parameters were critical or no direction as to which of many possible

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choices is likely to be successful. [citations omitted]. In others, what was "obvious to try" was to explore a new technology or general approach that seemed to be a promising field of experimentation, where the prior art gave only general guidance as to the particular form of the claimed invention or how to achieve it. [citations omitted]" *In Re O'Farrell*, 853 F.2d 894, 903 (C.A.F.C. 1988). As cited above, both the motivation to combine prior references and the expectation of success must be founded in the prior art.

In this case, the Patent Office provides motivation to combine the references as a desire to: (a) increase the efficiency of PHA production from yeast or to improve cost effectiveness of producing PHA (page 5 of the Office Action) or (b) to determine if genes involved in PHA synthesis have different transcript profiles under aerobic or anaerobic conditions (as stated at pages 5-6 of the January 2006 office action). With respect to argument (a): The record does not show any prior art suggestion that anaerobic conditions would increase efficiencies: withdrawal of this argument or clarification of this point is requested. Argument (b) is merely a suggestion that anaerobic fermentations should be tried to see what would happen: this is mere speculation about what scientists might like to try out and is not based on the prior art; indeed, one could make the assumption that scientists are curious about everything and that anything would be an interesting experiment: such reasoning would, respectfully, be absurd. Arguments that are not based on the prior art are mere hindsight and can not stand; therefore this argument (b) should be withdrawn.

Further, the Patent Office explains that argument (b) rests on the proposition that "Linde et al. teaches little difference of aerobic and anaerobic transcript profiles of *S. cerevisiae*" (page 7 of Office Action) and that an artisan would therefore be motivated to determine if genes involved in PHA synthesis have different transcript profiles under aerobic or anaerobic conditions". This statement by the Patent Office explicitly suggests that the references would be combined for the sake of making a determination as to what would happen; not only is this a plainly the impermissible "obvious to try" rationale, it is

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an admission that the cited references do not predict success for the claimed invention. If Linde et al. stands for the proposition that the profiles are expected to be the same under both conditions, then the very same reference would not motivate testing the profiles to see if they might turn out to be the same.

The suggested motivations to combine the references are only, at best, assertions that it would have been obvious to try to make the claimed invention. The rejections fall short of pointing to a reason for actually doing the experiments that the Applicants had to do to discover the claimed invention.

*Production of PHA by engineering of metabolic pathways is an unpredictable art*

The literature explicitly states that these are unpredictable arts such that there would be no reasonable expectation of success for making the claimed invention. Madison et al. state that "Taken together, these molecular genetic data provide a glimpse of the complexity of PHA metabolism. Since PHA formation is dependent on the fluxes in central metabolic pathways and the levels of precursors, a detailed knowledge of the molecular physiology of PHA metabolism is *critical* for successful implementation of transgenic PHA producers. Unlike the production of heterologous proteins, which relies mostly on sufficient gene expression, recombinant PHA production involves coordinated expression of heterologous enzymes over a prolonged period and with a concomitant redirection of the metabolism of the host. As a consequence of the metabolic changes introduced by expressing the *pha* and *phb* genes, the cell will induce its own responses, which are not necessarily favorable for PHA production. It is therefore *critical* to understand how bacteria normally regulate PHA formation and how undesired responses from a recombinant host can be prevented. *Only then* can recombinant processes be successfully developed and lead to what are expected to be the most efficient PHA production processes." Madison et al, at page 35 under "Conclusions", emphases added.

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These arts are related to metabolic pathway engineering and not to merely getting an organism to express some number of heterologous proteins. It is one thing to have a few heterologous proteins expressed but quite another to have any meaningful amount of PHA produced, e.g., the claimed at 1.5% of dry cell weight. The metabolic changes introduced by expressing the appropriate genes, the cell will induce its own responses, which are not necessarily favorable for PHA production. **Madison et al. explains that merely expressing certain genes does not provide even a *punctilio* of predictability for PHA production.**

For these reasons, the literature states that these are unpredictable arts such that there would be no reasonable expectation of success. Therefore the rejection of the claims can not stand.

*The teachings of Linde et al are irrelevant to predicting success of production of PHA in anaerobic cultures*

The teachings of Linde et al are irrelevant to predicting success of production of PHA in anaerobic cultures because Linde et al. is directed to the production of heterologous proteins, but what is claimed involves redirection of the metabolism of the host - not merely the expression of particular genes. PHA is an intermediate metabolite. Production of PHA depends on the presence of certain enzymes and also, among other things, a high ratio of NADPH to NADP (see discussion, below). Therefore the presence of certain enzymes is not an adequate basis to predict PHA production. The Patent Office has argued that Linde predicts the presence of certain enzymes. The Examiner's argument is not therefore not an adequate basis to predict PHA production: merely having certain enzymes present is not enough. As explained in the passage of Madison et al. quoted above, even if Linde et al. were assumed to predict the success of expression of certain proteins, **this would not be enough to predict success** because it does not speak to the critical aspects of the cell's metabolic pathways. Thus Linde et al. is largely

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irrelevant because it does not address factors that the literature states are critical to success. Accordingly, withdrawal of the obviousness rejection is requested on the grounds that no prima facie case of obviousness has been made.

*An artisan would not have reasonably expected anaerobic fermentation to be a successful process for PHA production. Further, the Applicant's claimed method is surprising and contrary to conventional wisdom.*

It is respectfully submitted that the arguments, above, provide multiple reasons for withdrawal of the rejection. Moreover, the Applicant is entitled to a patent unless there is evidence logically developed from the prior art for not granting the patent. Nonetheless, there are further aspects to the claimed invention that provide additional evidence of its patentability. One aspect is that an artisan, before reading the Application, would not have reasonably expected anaerobic fermentation to successfully produce any appreciable amount of PHA, i.e., the claimed at least 1.5% PHA per dry cell weight.

Among other factors, the ratio of NADPH to NADP is key for PHA production, with a high ratio favoring PHA production.<sup>1</sup> NADPH is formed primarily through operation of the pentose phosphate cycle and the prior art does not indicate how much of the metabolism is directed into this cycle, or how to reliably predict how this metabolism will be directed.

Moreover, as explained in the section of the Application entitled "Transhydrogenase Systems" on page 21, cellular metabolism must maintain the redox balance of the cell if the cell is to survive. In aerobic fermentation processes, oxygen is available to serve as an electron sink to carry away excess H ions in the form of water. This prevents electrons from building up in the electron transport system. In anaerobic

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<sup>1</sup> "The availability of reducing equivalents in the form of NADPH is therefore considered to be the driving force for P(3HB) formation." Linde et al., page 27, last sentence of first paragraph.

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processes, however, the elimination of the H ion is much more problematic. The production of ethanol in yeast is driven by the need to "find" an electron sink that is an alternative to oxygen. The cell's change from aerobic to anaerobic culture is a dramatic change that affects its metabolic pathways in many aspects. While many proteins essential to cell survival might be expressed at the same levels, the metabolism of the cell has to be significantly re-oriented to cope with a lack of oxygen. PHA and ethanol are both metabolites and not heterologous proteins expressible by the familiar mechanisms of genetic engineering. When culture conditions are dramatically changed, predicting which metabolites will be favored is quite problematic, as explained in Madison et al., quoted above.

In the case of PHA production, it was quite problematic to contemplate successfully producing a metabolite that depends on NADPH/NADP ratios under conditions that are known to re-orient the cells to offload excess H ions as ethanol, i.e., in anaerobic culture. As already stated, it was quite unpredictable. In this case, when the cell's metabolic machinery is dedicated to making ethanol for survival, trying to intervene in that path to divert resources to making PHA seemed counterintuitive. And, crucially, yeast cells must maintain a delicate balance between NADPH and NADH production and consumption to maintain their redox balance. While many cells have a transhydrogenase system that permits interconversion between NADPH and NADH, it is conventionally believed that yeast do not have this capability. See Application, "Transhydrogenase Systems" on page 21.

Surprisingly, however, the results in the Application suggest that PHA can serve as a sink for electrons (NADH) during anaerobic metabolism. This is in contrast to a normal fermentation product that is excreted from the cell. Conventional wisdom provides no prediction of this capability and has no ready explanation. Evidently, however, PHA can substitute for a normal fermentation product and accumulate as a fermentation product substitute within the cell. This result implies at the same time that

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there is a mechanism to convert the excess NADH into NADPH to enable PHA synthesis, despite the fact that there is no conventionally known transhydrogenase system for their interconversion. Alternately this could indicate that perhaps NADH can be directly used for intracellular PHA formation. In hindsight, using PHA as an electron sink seems like a "smart" move for the yeast cells, but of course the question was never how "smart" the cells were, but if their metabolic machinery could possibly tolerate PHA production. For all of these reasons, an artisan would not have reasonably expected anaerobic fermentation to be a successful process for PHA production.

The Office Action's response to Applicant's arguments.

The Office Action made some responses to on Applicant's arguments starting at page 6 therein. The Office Action argued that Linde et al. teaches that yeasts are unique in exhibiting fast growth in the presence or complete absence of oxygen such that there would be a motivation to use transgenic yeast so as to permit flexibility and thereby improve cost effectiveness of producing PHA. Respectfully, however, this argument does not speak to Applicant's point about Linde et al., which is that Linde et al. is directed to the production of heterologous proteins, but what is claimed involves redirection of the metabolism of the host - not merely the expression of particular genes, as discussed in detail herein.

The Office Action argued that an artisan would, in fact, expect successful anaerobic production of PHAs (page 6 Office Action), and points to Lee et al. (Waste Management 19 (1999) at page 134 therein. Respectfully, Lee et al. stands for the proposition that what is apparently anaerobic synthesis of PHA can take place in consortia of bacteria such as in the activated sludge of wastewater plants. To conclude from this that yeast can make PHA anaerobically is not a fair inference since the poorly understood activity of bacteria cannot be fairly attributed to yeast, which are different

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organisms, and since it is necessarily unknowable if a not-understood mechanism would be operable in a much different setting, namely, yeast cultures. In fact, anaerobic synthesis has not been studied in pure cultures and therefore its mechanism and regulation is not well understood.

Objective evidence in support of these facts is provided by the attached peer-reviewed publication entitled Kinetic Studies and Biochemical Pathway Analysis of Anaerobic [PHA] Synthesis in *Escherichia coli*:

"PHA production under conditions of oxygen stress and in the absence of oxygen has been reported for organisms that natively accumulate PHA (3, 4, 45). Anaerobic PHA production has been studied in undefined bacterial consortia found in a wastewater treatment process known as enhanced biological phosphorous removal (for recent reviews, see references 31 and 46). These studies examined a still unclear relationship between a PHA-accumulating bacterial consortium and the removal of phosphorous compounds from wastewater streams (37)." Carlson et al., *App. Env. Microbiol.*, Feb 2005, 713-720 (2005), at page 713, third paragraph.

Significantly, the last paragraph of the Discussion section of Carlson et al., see *App. Env. Microbiol.*, Feb 2005, 713-720 (2005), points out that yeast lacks a transhydrogenase system that would convert NADH into NADPH. Under anaerobic growth conditions, NADH accumulates in the cells but it is well-known that NADPH is needed for PHA synthesis. As already stated, the Applicants in the instance case have discovered that there is a mechanism to convert the excess NADH into NADPH to enable PHA synthesis, despite the fact that there is no conventionally known transhydrogenase system for their interconversion. This fact underscores the differences between the claimed yeast systems and the bacterial systems cited in the Office Action.

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Respectfully, the Office Action's reliance on a poorly-understood bacterial consortium to predict yeast function underscores the weakness of the Patent Office's case; no artisan of ordinary competence would accept the assertion that such a system would have useful predictive value for the claimed yeast.

Another point made in the Office Action is that absolute certainty is not required to combine the references. This point is acknowledged. The record, however, including Madison et al., **plainly states** that PHA metabolism is an unpredictable art.

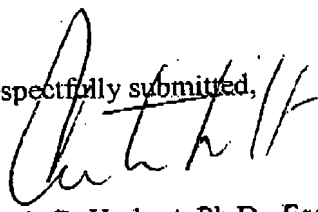
The Office Action suggests that Applicants provide no evidence that these are unpredictable arts. The Applicants responses, however, make multiple specific point cites to publications that plainly state the same: this evidence is of-record and can not be ignored or dismissed as non-evidence. Moreover, the results set forth in the Application suggest that PHA can serve as a sink for electrons (NADH) during anaerobic metabolism, which is surprising, as explained in the Application for the reasons provided above. These facts have not been disputed in the Office Action.

#### Request for Allowance

In view of the foregoing, it is submitted that this application is in condition for allowance. Favorable consideration and prompt allowance of the application are respectfully requested.

The Examiner is invited to telephone the undersigned if the Examiner believes it would be useful to advance prosecution.

Respectfully submitted,

  
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